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HISTAMINE INDUCED CHANGES IN THE SULPHATE METABOLISM OF THE DUODENUM AND PREPYLORIC REGION OF THE STOMACH: AN EXPERIMENTAL STUDY WITH SPECIAL REFERENCE TO ULCER FORMATION

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PREFACE.

The present study was carried out in the Department of Physiology of the University of Turku. My former teacher, Professor K. HARTIALA, M.D., Head of the Department, made it possible for me to undertake the investigation, and encouraged me by freely discussing and criticizing my work. I wish to express my deep gratitude to him.

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Turku, December 1960.

Ilkka Häkkinen.

I

INTRODUCTION.

During recent years the volyme of literature dealing with the problem of peptic ulcer has increased greatly — an indication that this problem is far from being solved.

The so called "acid theory" cannot account for all the findings which have been recorded. In particular it does not explain the observed differences between gastric and duodenal ulcers, the most important of which appears to be the difference in the amounts of hydrochloric acid secreted in the two conditions. In order to account for this difference "vascular" or "tissue" factors have sometime been invoked, especially to explain the formation of some experimental ulcers. Evidence that such factors exist is so far mostly histological, and no convincing progress has been made towards their definition.

There is good clinical and experimental evidence that the presence of hydrochloric acid is essential to the formation of a peptic ulcer, to the extent that many scientists now consider the essential rôle of acid to be established; the part played by a tissue factor is still problematical. The purpose of this work is to try, under conditions as simple and as nearly physiological as possible, to find out in what way the state of the gastric or duodenal tissue differs from normal at the earliest stage in the formation of an ulcer.

The earliest change to be observed histologically in the area where an ulcer is about to form resembles a non-specific mesenchymal inflammatory reaction. There is experimental evidence that such a reaction is reflected in an alteration in the sulphate content of the tissue, at least when this tissue is mesenchymal — acting on the hypothesis that such tissues in the gastric and duodenal wall can respond directly to stimulation and do play an important part in resistance and healing ability, I have set out to investigate their sulphate content. When there is any variation from normal, it has had to be decided whether it caused by a stimulus from the lumen of the gut — in other words hydrochloric acid — or whether there is a primary tissue factor which is independent of the presence of hydrochloric acid, and which may play a part in the formation of an ulcer.

II

REVIEW OF THE LITERATURE.

Histamine, as is well known, can be found in almost every part of the body. Its main action is thought to be upon the circulation. Traumatic and anaphylactic shock have also been attributed to the action of histamine. It has been thought that histamine plays an essential part in the stimulation of gastric secretion, and that this is one of its most important functions. There are contrasting views, and some maintain that histamine is physiologically of minor importance. As they point out, intensive work for several decades has failed to find a sure function for histamine. All agree that histamine has great biological activity under experimental conditions, and interest in it remains active for this reason. The dicussion which follows is concerned only with the possible relevance of histamine to the ulcer problem; and will deal with the effect of histamine upon gastric secretion, the origin of "active" histamine, or histamine that can be released by histamine liberators, the localisation of histamine in the wall of the stomach and duodenum, its vascular effects, and our knowledge of artificial histamine ulcers.

The discussion will then be concerned with the fundamentals of sulphate metabolism in connective tissue and epithelium, and the possibility of distinguishing between the mesenchymal reaction to non-specific trauma and specific ulceration as provoked by histamine.

HISTAMINE, ITS OCCURRENCE AND FUNCTIONS.

1. THE ISOLATION OF HISTAMINE IN THE STOMACH.

EDKINS (1906) showed that the mucosa in the pyloric antrum contained a substance which stimulated the secretion of acid. Injection of an extract of pyloric mucosa caused an increase in the rate of secretion of acid. It was later proved in experiments using dogs with isolated gastric pouches that there was a humoral mechanism causing secretion of acid (IVY and FARRELL 1925). Analysis of extracts of gastric mucosa led to the finding of a substance which resembled histamine. SACKS et al. (1932) showed that this substance was destroyed by histaminase. It was also shown to affect the circulation, and ultimately histamine itself was separated from such extracts.

2. THE EFFECT OF HISTAMINE ON GASTRO-DUODENAL SECRETION.

It has been shown long ago that histamine when administered parenterally stimulates gastric secretion (POPIELSKI 1920, BEST and MCHENRY 1931). CODE (1956) described histamine as "the universal gastric secretory stimulant". Sensitivity to histamine varies greatly in different species of animals. Cats, dogs, and guinea pigs differ from rats and mice. The dose of histamine has to be much greater in the latter (KOMAROW et al., 1944; DAVENPORT and CHAVRÉ 1950). It is therefore important in the experimental use of histamine to suit the dose and the method of administration both to the species of animal used and to the physiological effect which it is designed to have. For instance, too large a single dose given over a short period may cause inhibition of the secretion of acid and not stimulation (POPIELSKI, 1920; ROTHLIN and GUNDLACH 1921—22).

HANSON, GROSSMAN and IVY (1948 a) have studied the least and the greatest effective single doses of histamine in dogs and in man. In

dogs the least dose which would increase the rate of secretion of hydrochloric acid was 0,045 mg/kg body weight. To ensue maximal secretion 1,8 mg/kg is necessary. Man was found to be about ten times as sensitive to a small dose, in that 0,004 mg/kg body weight was the minimal effective dose. Maximal secretion have been effected by 0,4 mg/10 kg body weight (KAY 1953), LANE, IVY and IVY (1957) have pointed out that the absolute dose of histamine which causes a minimal increase in the rate of secretion of acid is of a similar order in man, dogs, cats and rats, quite irrespective of their size.

When the maximal dose of histamine is exceeded, histamine is found to have other and most interesting effects upon the stomach and duodenum. It should be stressed at this stage that the dose of histamine which produces a minimal response from the parietal cells is very small, and causes no measurable increase in the blood concentration (IVY, GROSSMAN and BACHRACH 1951). Ivy regards the parietal cells of the stomach as the most sensitive known indicators of the presence of histamine.

GILMAN and COWGILL (1931) have examined, using dogs, the effect on the secretion of a gastric pouch of a single subcutaneous dose of histamine. The rate of secretion of acid increases immediately after the injection, reaches a maximum during the first hour, and decreases to its original value between 2 and 2½ hours after the injection. Thus the effect of a single dose is transient, and indicates that histamine is rapidly absorbed and rapidly eliminated. The continuous administration of histamine has been desirable in many forms of experiment, and continuous intravenous infusion has often proved difficult. The discovery of CODE and VARCO (1940) has opened up entirely new experimental possibilities. The intramuscular administration of histamine in powder form suspended in a mixture of beeswax and mineral oil made it possible to produce a protracted and constant hypersecretion of acid. Absorption was delayed, and a slight increase in the level of histamine in the blood could be maintained for about 24 hours after a single injection. During this period the Heidenhain pouch of a dog secreted 675 ml of gastric juice at an acidity only a little less than the theoretical maximum of 150 mEq/l. During a control period of 24 hours the secretion was 15 ml. 30 mg of histamine were given intramuscularly, as two simultaneous injections of 15 mg. A similar dose injected in water causes an increased secretion lasting only 4 ½ hours (CODE and VARCO 1942).

Pepsin is secreted in the stomach, as well as acid. It is secreted continuously, and at a fairly constant rate (LINDE 1950; JANOWITZ and HOLLANDER 1952). After an injection of histamine, the rate of secretion of pepsin at first rises and then falls abruptly (GILMAN and COWGILL 1931). The rise is thought to be due to a washing out of pepsin from the glands by the flow of acid, and therefore to be apparent rather than real. The total quantity of pepsin secreted is not affected by histamine (BABKIN 1930; LINDE 1950).

BACHRACH, GROSSMAN and IVY (1946) state that the secretion of pepsin is in practice always adequate and that it is the secretion of hydrochloric acid which is critical. The character of the response to histamine suggests that it is the immediate stimulus to the secretion of hydrochloric acid.

Gastric juice always contains mucus. It is secreted continuously, but the rate of secretion varies and is influenced by the presence of acid in the stomach, mechanical irritation, and the movements of the stomach itself (IVY, GROSSMAN and BACHRACH 1951).

HARTIALA, KASSINEN and SUUTARINEN (1954) have examined the effect of histamine on the secretion of an isolated duodenal pouch. They showed that in five out of six animals a single subcutaneous injection of histamine in water caused a definite rise in the rate of secretion of mucus from the pouch. Repeated injections did not usually cause this rise to be maintained. The results were not consistent, since two dogs showed no continued response at all, while in one the rate rose in 4—6 days, and did not fall to its previous level until administration of histamine stopped. The authors concluded that the effect of histamine was indirect, and that an increased secretion of HCl caused a humoral stimulation of the duodenal glands. Were this so, one would have expected more constant and sustained response, since the secretion of the duodenal glands is not easily exhausted. STEVENS (1935—36) found in dogs that the duodenal secretion was able in 24 hours to neutralize completely 1800 ml of 0,5 % HCl — twice the maximal secretion which CODE had managed to produce by the intramuscular injection of histamine in beesxax (CODE and VARCO 1942). It would be interesting to repeat HARTIALA's experiment after diversion of acid from the duodenum.

3. THE MECHANISM BY WHICH HISTAMINE CAUSES THE SECRETION OF ACID.

It is thought that histamine has a direct action upon parietal cells. IVY and FARRELL (1925) showed that the gastric mucosa would respond to histamine even after being transplanted into the skin. More recently is has been shown that direct perfusion of gastric mucosa in vitro will also excite secretion (DAVIES 1946, 1948; DAVENPORT 1950). More recently still, experiments have been carried out using C^{14} labelled histamine. The labelled histamine did not enter the lumen of the stomach (SCHAYER and GROSSMAN 1956), although after the injection of histamine free histamine could be detected in the gastric juice. The quantity of histamine in gastric juice is closely related to the volyme of gastric juice secreted (MACINTOSH 1938; EMMELIN and KAHLSON 1944; CODE, HALLENBECK and GREGORY 1947). It thus seems that histamine, at least as it appears in the gastric lumen, is endogenous. Despite all the research of recent years the mechanism by which histamine affects the parietal cells is not known: how, for instance, does histamine enter the lumen of the stomach, and what part does it play in disturbances of gastric secretion in man, and in the production of histological lesions in the distal stomach and duodenum?

4. VASCULAR ACTIONS OF HISTAMINE.

BURN and DALE (1926) studied the effect of histamine on the blood vessels of the cat, and observed that they became dilated: adrenaline or pituitrin, on the other hand, caused constriction. Dogs and apes responded in the same way as cats to all three substances. When the arterioles of a cat were perfused with histamine they contracted, while those of a dog dilated. Moreover, an extremely small dose of adrenaline caused relaxation rather than contraction, perhaps because it released histamine, while a very small dose of histamine caused vasoconstriction, since it released adrenaline. These authors' hypothesis have not been accepted by later workers. Intravenous injection of adrenaline causes peripheral vasodilatation — in the hand for instance —, while if the adrenaline is injected directly into the brachial artery vasodilation does not occur. WHELAN (1952; 1956) has investigated the possibility

that this vasodilatation is caused by histamine. He excluded the possibility that the effect of intravenous adrenaline could be due to circulating histamine. Moreover, although the vasodilatation produced by intraarterial injection of histamine could be prevented by antihistamines (DUFF and WHELAN 1954), antihistamines had no effect upon adrenaline-induced vasodilatation. It is thus clear that histamine plays no part in the vasodilatation caused by adrenaline. DALE (1948) has suggested that "intrinsic" histamine is liberated within the effector cells — in this case the smooth muscle of the vessel walls — and that antihistamines are unable to penetrate these cells.

Study of the direct reaction of the blood vessels of the wall of the stomach and duodenum to histamine is complicated by the effect of the hydrochloric acid that is secreted. The stomach and duodenum have a rich vascular supply with some features peculiar to this area, in particular the spiral end-arteries of the superficial layers of the mucosa. These vessels are able to adapt to any variation in the thickness of the mucosa caused by stretching or movement. There are also arteriovenous anastomoses along the vessels and in the muscularis mucosae, which may serve on occasions to shunt a large proportion of the circulating blood away from the mucosa.

EPPINGER and LEUCHTENBERGER (1932) gave histamine to dogs and observed that the first effect was oedema of the gastric mucosa. They therefore attributed to histamine angiotoxic effects and the ability to cause necrosis. Any haemorrhages seen in the mucosa were a secondary phenomenon due to digestion by HCl-pepsin. MERKEL (1942) gave histamine (0.13—0.28 mg/100 g body weight in water) to eleven guinea pigs every half-hour for eleven hours. He observed microscopically severe oedema extending down to the serosa, and necrosis and haemorrhages on the mucosal surface. If the stomach were drained and bathed with sodium bicarbonate, only oedema could be seen. MERKEL considered that the primary effect of histamine was circulatory, and due to dilatation and increased permeability. Hydrochloric acid was thus enabled to enter the capillaries and produce thrombosis and acid haematin, and on this basis ulceration might occur. HEINLEIN and KASTRUP (1938) observed that in the cat, too, the primary effect of histamine was oedema. Subepithelial lesions suggesting an angiotoxic effect were found in the duodenum as well as the stomach. Macroscopically the gastric mucous membrane was swollen, and in the antrum and pyloric canal there were pin-point

haemorrhages. Similar changes could be seen in the duodenum. HEINLEIN thougt that the vascular and angiotoxic effects of histamine were primary, and impaired the vitality of the mucosa, so rendering it vulnerable to the effect of HCl and pepsin. He did not study the effect of histamine after elimination of the effect of HCl.

KOWALEWSKI (1954) gave histamine intramuscularly (75 mg/kg body weight) to guinea pigs with phenergan 30 mg/kg subcutaneously; after 48 hours all the animals showed congestion and ulceration in the duodenum as well as the stomach. Pituitrin is known to have a vasoconstrictor action: KOWALEWSKI succeeded in preventing histamine ulcers in guinea pigs by the simultaneous administration of pituitrin. In another paper on the same subject (KOWALEWSKI and BAIN 1954) he managed to prevent histamine ulceration in 86 % of the animals. He considered that pituitrin prevented the vasodilator effect of histamine. He later (KOWALEWSKI et al. 1958) studied the effect of the same substances in the dog. Repeated injections of histamine caused ulcers in all the animals; when pituitrin was given as well the rate of ulceration was 16 %. In acute experiments on pouch dogs he observed that histamine alone caused congestion, while pituitrin alone caused pallor and ischaemia. The two together left the mucosa apparently normal.

BARONOFSKY and WANGENSTEEN (1946) considered whether the effect of histamine could be reproduced by other substances. They observed that dogs given histamine in beeswax, 30 mg daily for six days, did not develop ulcers. If they were given nitroglyserine in beeswax at the same time the ulcer frequency was 100 %. The same results were obtained in rabbits. The authors concluded that nitroglyserine caused anoxia of the mucosa by reduction of the tone of the splanchnic vessels and slowing of the circulation in them, and that the additive effects of nitroglyserine and histamine favoured the concept that histamine had an angiotoxic action. ROTH and IVY (1944, 1945) caused ulceration in cats by giving caffeine in beeswax, and also injected caffeine intravenously. They suggested that the action could be separated into five components, hypersecretion, vasodilatation, congestion, hypermotility and toxity to cells. They also suggested that the vascular components weakened the mucosa and made it vulnerable to the other effects.

5. THE METABOLISM OF HISTAMINE.

A. General.

Exogenous histamine is not thought to be bound in the body (SCHAYER 1952; SCHAYER and SMILEY 1954): it is oxidized and so inactivated and eliminated. There are many experimental findings which suggest that histamine is rapidly eliminated from the body. For example, the injection of histamine in water is only effective in increasing the secretion of acid for 2 hours. If the effect is to be prolonged, repeated or continuous injection or injection of a depôt preparation are necessary. Histamine may be formed in the tissues from the essential aminoacid l-histidine by the action of histidine decarboxylase. SCHAYER, SMILEY and DAVIES (1954) observed that histamine was formed in rat's skin at a rate of 2 μg/g of skin per day. If a histamine liberator, 48/80, were given, the formative capacity of the skin was much increased. It has also been observed that the stomach and intestine can form histamine from histidine in vitro (SCHAYER 1956) and in vivo (SCHAYER and IVY 1958). The rat differs from most other animals in its metabolism of histamine (CODE 1956); GADDUM (1956) found that cats and dogs could not synthesize histamine, and suggested that synthesis took place by bacteria in the intestine. More work is needed before this suggestion can be regarded as established. PERRY (1956) and BROCKLEHURST, HUMPHREY and PERRY (1955) observed that the large concentrations of mast cell histamine in the skin of the rat could be lowered by the action of 48/80 to the concentration normally present in guinea pig's skin. Cortisone markedly inhibits the conversion of histidine into histamine (SCHAYER, SMILEY and DAVIES 1954).

RILEY and WEST (1953 a and b); RILEY (1954); CASS et al. (1954) and GRAHAM et al. (1955) showed that the concentration of histamine in tissue was closely related to the number of mast cells present. After the discovery of histamine liberators by MACINTOSH and PATON (1949), it was possible to prove with their help that mast cells in fact contained histamine (RILEY 1953; FAWCETT 1954 and MOTA, BERALDO and JUNQUEIRA 1953). MOTA and VUGMAN (1956) studied the effect of 48/80 on various tissues of the guinea pig, and found that under experimental conditions this species did not liberate histamine as did the rat or the dog. MOTA, BERALDO and JUNQUEIRA (1953) claimed that both 48/80 and stilbamid, another histamine liberator, cause extensive loss of

granulation in mast cells. This effect occurs in vitro, but is inhibited by the addition of heparin to the system. WERLE (1956) succeeded in binding heparin and histamine in vitro in relative proportions similar to those in which they occur in the tissues. SMITH (1958 b) investigated the action of the best known histamine liberators, 48/80, stilbamid, protamine sulphate and toluidine blue on mast cells. Toluidine blue caused the cells to swell to one third more than their original size, and their granules to stain metachromatically and then vanish. The author concluded that histamine was liberated from a hitherto osmotically inert state where it was combined with heparin. As a result water entered the cell; when histamine left the cell the water left also. Another possible explanation of histamine liberation comes from the demonstration that 48/80 and some antigen-antibody reactions liberate histamine from mast cells by activating a lytic enzyme. Decylamine, on the other hand, causes changes in the cell membrane and consequent disintegration of mast cells, without an enzyme being involved (HÖGBERG and UVNÄS 1957, 1958, 1960). Under experimental conditions 48/80 only releases histamine from some 60 % of mast cells (UVNÄS and THON 1959). UVNÄS considers that the same may happen in vivo, quoting as evidence the impossibility of wholly freeing the skin of a rat of histamine by giving 48/80.

B. The metabolism of histamine in the stomach and intestine.

IMSCHWEILER (1940) studied the alterations in the basophilic granular cells of the stomach of the rat which followed the giving of histamine. He observed that histamine, whether given into the stomach by tube or by subcutaneous injection, increased both the number and the granularity of such cells. BORBOLA, BIKICH and FAREDIN (1955) found that all of a dose of histamine injected into the gastric artery reached the gastric vein, or, if the vein were tied, the gastric lumen. According to SCHAYER and GROSSMAN (1956), injection of C14 labelled histamine does not lead to the appearance of labelled histamine in the lumen of the stomach, although the concentration of histamine in the lumen rises. CODE (1956) considers that histamine which enters the gastric lumen is endogenous and derives from the mucosa itself. SMITH (1953), thought that histamine liberated into the lumen after administration

of curare or 48/80 was at least partly derived from elsewhere in the body. One proof of the effectiveness of endogenous histamine from elsewhere in the body is the increase in the rate of secretion of hydrochloric acid which results from hard rubbing of the skin in patients with dermatographia, when the concentration of histamine in the blood can be shown to increase (KALK 1929; ROSE 1941). In the rat 48/80 does not liberate histamine in the stomach or duodenum, although it does in the skin (FELDBERG and TALESNIK 1953). MOTA et al. (1956) noticed that there was a discrepancy between a high concentration of histamine and a very small number of mast cells in the gastric fundus and duodenum of the rat, and observed that 48/80 could not change the concentration of histamine under such conditions, as it could where the concentration of histamine was related to the number of mast cells. It thus appears that in the stomach and duodenum histamine does not originate in mast cells. MONGAR and SCHILD (1952) showed that in the guinea pig histamine in the stomach and duodenum did not derive from mast cells. RILEY and WEST (1953 b) found that in the cat 48/80 lowered the concentration of histamine in the stomach, but not elsewhere in the intestine. According to MOTA, FERRI and YONEDA (1955) the body of the stomach contains a considerable number of mast cells.

HILL and CODE (1959) have recently studied the effects of meals, of hypoglycaemia, and of the injection of histamine and of cortisone upon the concentration of histamine in the gastric mucosa. Only hypoglycaemia caused the concentration of histamine to rise, and even then the rise was limited almost entirely to the antrum. No other agents tried affected the concentration of histamine at all. They suggested that the amount of free histamine released at any one time was in any case small and was corrected so fast that no change in concentration could be detected. Against this view, their own demonstration of the effect of hypoglycaemia shows that the synthesis of histamine can be very rapid.

The localisation of histamine in the stomach has been defined accurately: FELDBERG and HARRIS (1953) have drawn a profile showing the concentration of histamine in the different layers of the dog's stomach. They cut horizontal sections from different levels of the wall of the stomach and intestine, and compared the results of their analysis and of microscopical examination of duplicate samples. In the body of the stomach high concentrations of histamine were found in the area occupied by parietal cells and in the muscularis mucosae; the sub-

mucosa also contained some histamine. The authors thought that the histamine in the parietal cell area was not contained in mast cells, but that in the submucosa it might be. In the pyloric antrum a high concentration of histamine was found only at the level of the pyloric glands. In the duodenum it was found in a high concentration at two levels. Such an analysis has not been carried out in guinea pigs, but the same author (FELDBERG 1956), analysing the whole mucosa together, found in guinea pigs a low concentration of histamine in the wall of the stomach, and a moderate concentration in the duodenal wall. The values were lower than those at a comparable site in the dog.

TRACH, CODE and WANGENSTEEN (1944) have measured the concentration of histamine in the fundus of the stomach in patients suffering from gastric and duodenal ulcers and from gastric carcinoma, but found no constant differences between them. The mast cells in the wall of the human stomach have been counted by QUENSEL (1933) in patients with carcinoma of the stomach, and by SUNDBERG and SIURALA (1959) in patients with ulcers of the stomach or duodenum and with carcinoma: again there was no difference between the patients.

BORBOLA, BIKICH and FAREDIN (1955) estimated the concentration of histamine at different levels in the walls of gastric and duodenal ulcers, and found it usually to be higher than in healthy mucosa: the highest concentrations were found in the proliferating submucosal tissue, and in gastric rather than duodenal ulcers. Neither carcinomata nor carcinomatous ulcers contained high concentrations. Measurement of the concentration of histamine in granulating scars of the skin showed it to be no higher than in normal skin elsewhere. The possible significance of this localisation of histamine is still unknown. There is evidence, from work published many years ago, that in the cat injection of histamine in water directly into the wall of the stomach causes local ulceration, whereas no ulceration ensues if the histamine is injected subcutaneously (O'SHAUGHNESSY 1931).

6. HISTAMINE AND EXPERIMENTAL ULCER.

The first experiments designed to produce histamine ulcers were carried out in rats: the animals were fasted for 24 hours before the histamine was given: after it was given their treatment varied (BÜCHNER

and Molloy 1927; Büchner, Siebert and Molloy 1928—29). Rats which were given 14 injections of histamine and were fasted for a day after each injection developed ulcers. The authors concluded that the mucosa was more vulnerable to the acid secreted in response to histamine when the stomach was empty than when the stomach contained food. Bürkle de la Camp (1929) confirmed their results: in his experience an unwonted fast in combination with an injection of histamine regularly caused ulceration in rats. Intravenous injection of histamine in cats does not cause ulceration, but did cause histological changes in the mucosa (Heinlein and Kastrup 1938). It thus appears that the effect of a single intravenous dose of histamine was too short-lived to produce ulceration. Merkel (1942) managed to produce changes in the stomach in guinea pigs by single and by repeated doses of histamine. The earliest lesions were in the body and fundus of the stomach, while the duodenum remained unaffected: in this chronic experiment 44 guinea pigs received a subcutaneous injection of histamine in water every day for about 80 days. The dose given varied between 0.18 and 0.45 mg/100 g body weight.

It is most important to ensure the correct experimental conditions if histamine ulceration is to be constant in its incidence. The contrasting results of experiments on dogs illustrate this very well. Gage, Ochsner and Hosoi (1936), using histamine in water, claimed to have produced erosions and ulcers. Overgaard (1931) and Orndorff, Bergh and Ivy (1935) failed to do so, although they injected histamine repeatedly over a period extending into months at a dose of 0.5—2 mg daily.

Histamine in beeswax regularly causes ulcers in most laboratory animals, even after a short period (Walpole et al. 1940; Varco et al. 1941; Hay et al. 1942; Shoch and Fogelson 1942; Grossman, Dutton and Ivy 1946). The ape and rabbit are the most resistant (Hay et al. 1942). Guinea pigs usually develop duodenal ulcers, which often perforate (Hay et al. 1942). In the opinion of Code (1943) it is the hydrochloric acid secreted in response to the continuous stimulation which is immediately responsible for the ulceration. Hanson, Grossman and Ivy (1948 b), in dogs, found that continuous intravenous infusion of histamine or repeated subcutaneous injections for 2—16 days regularly caused both gastric and duodenal ulcers: the dose used was that necessary to cause maximal secretion. If then the dose was increased further, ulceration occurred sooner and was more severe. Duodenal ulcer has been reported in man following therapeutic infusion of histamine (Mc

Hardy and Browne 1944; Iams and Horton 1946). The ulcers healed rapidly when histamine was withdrawn.

Olovson (1950) studied the earliest histological changes in the gastric mucosa following the injection of histamine. Haemorrhage was the earliest change seen. Williams (1951), using guinea pigs, considered that a focal necrosis — due in his opinion to ischaemia — was the earliest change, and could appear in less than 15 hours. Such lesions were commoner in the stomach than in the duodenum. Watt (1959) examined the stomachs of guinea pigs under anaesthesia during the period following injection of histamine. He distinquished 4 stages: 1. an initial phase of ischaemia with consequent necrosis, 2. an occasional retrograde flow of blood followed by stasis, 3. haemorrhagic infarction, 4. dissolution of the infarct leading to ulceration. To blame ulceration entirely on the effect of an enhanced secretion of HCl appears to be an oversimplification. In both acute and chronic experiments the earliest changes following the injection of 2—10 mg of histamine in water have been in the fundus and body of the stomach, rather than the lesser curvature.

Brun (1952) gave large doses of histamine to guinea pigs and covered them with phenergan: a dose of between 20 and 50 mg constantly resulted in a 50 % incidence of ulceration. 100—350 mg caused perforation in every animal within 5 hours; such a large dose in fact does not cause a maximal secretion of acid. It therefore appears that the histamine must have had a direct effect on the tissue. Hoeven (1956) gave histamine in beeswax to guinea pigs once a day for 2 days, and found lesions both in the acid-bearing area and in the duodenum. Continuous instillation of 0.5 % HCl into the stomach for 48 hours caused some lesions, but they were very much less severe than those caused by histamine. Bachrach, Grossman and Ivy (1946) showed that application of 0.1 N HCl caused superficial necroses only. If part of the mucosa were excised the underlying connective tissue was not digested, and the wound healed rapidly. McIlroy (1927—28) found on the other hand that mucosal defects caused by operations on a dog's stomach did not heal normally if the animal were injected on alternate days with histamine in water. One must recall that in normal dogs the duodenal secretions are capable of neutralising very large amounts of hydrochloric acid (Stevens 1935—36). Cummins, Grossman and Ivy (1948) observed that instillation of 0.15 N HCl at a rate of 8—10 ml/kg in an hour caused ulcers in all the dogs tested. If the animals were

transfused at the same time with alkali, ulceration did not occur. The instillation of so much acid had produced a pathological acidosis in the animals and had affected the normal defence mechanism of the stomach and duodenum.

KITTLE, BATCHELDER and SCHAFER (1951) performed a total gastrectomy on dogs, and then gave them histamine in beeswax: none developed ulcers. This finding shows that acid plays a important part in the causation of histamine ulcers. JOHNSTONE (1958a, b) excised the parietal cell area of cats' stomachs, using an operation which preserved the other layers of the stomach wall. Histamine in beeswax was incapable of causing ulceration, provided that excision had been radical enough. He did not test the effect of acid or of histamine in combination with acid. An interesting observation has been made by HALLENBECK and JORDAN (1952). They had wiped the duodenal fistulas of dogs, gently, and apparently without causing any damage to the mucosa. In dogs which had received histamine in beeswax, circular ulcers appeared within 48 hours in the area which had been wiped. The duodenal mucosa was seen to be oedematous and easily traumatised in dogs receiving histamine.

Antihistamines do not prevent the effects of histamine on the stomach (FRIESEN, BARONOFSKY and WANGENSTEEN 1946; SANGSTER, GROSSMAN and IVY 1946; CRANE, LINDSAY and DAILEY 1947; WINTER and MUSHETT 1948). They have proved useful in experiments where histamine is used, since they counteract the other effects of histamine. It has already been described how 48/80 does not cause any change in the concentration of histamine in the stomach or intestine (MONGAR and SCHILD 1952). As SMITH (1958 a) has shown, it will cause ulceration in both the rat and the guinea pig, presumably because release of histamine from mast cells elsewhere in the body imitates the effect of an injection of histamine.

In summary, the following factors may play a part in the formation of histamine ulcers:

1. Continuous secretion of acid.

2. Impaired neutralisation, due either to a failure of duodenal secretion to keep pace with the increased secretion of acid, or to fasting.

3. Vasodilatation, statis and oedema.

4. The toxic effects of histamine.

It is hard to suggest any order of descending importance for these factors, since the last two are still speculative.

Although no change can be demonstrated in the concentration of histamine in the stomach wall after the injection of either histamine itself or 48/80, the fact that 48/80 causes ulceration indicates that circulating histamine does exert an effect on the parietal cells. Any change in concentration must be so transient and small as not to be measurable. It may be that the histamine normally present in the wall of the stomach has a physiological function of its own, and that circulating histamine is necessary to stimulate secretion. Since C14 labelled histamine does not appear in the gastric lumen after injection, circulating histamine can only be indirectly responsible, by displacement of histamine already present in the gastric wall, for the rise in the intraluminal concentration of histamine that takes place after its injection. Such a displacement might be compensatory in nature, and ensure a constant concentration of histamine in the wall of the stomach. When "free" histamine has again disappeared, the normal concentration might be quickly restored by synthesis from histidine. The observation that the stomach wall can synthesize histamine from l-histidine is compatible with such a hypothesis.

EXPERIMENTS ON SULPHOMUCOPOLYSACCHARIDES.

1. GENERAL EXPERIMENTS.

DZIEWIATKOWSKI (1949) injected radioactive sulphate intraperitoneally into rats, and followed its elimination. 67 % appeared in the urine within 24 hours, and 95 % appeared in the urine or faeces within 5 days. He observed that different organs differed in their ability to retain ^{35}S. Excretion was slowest from the bones and bone marrow. More discriminating experiments showed that the ^{35}S in rat cartilage was incorporated into chondroitin sulphuric acid (DZIEWIATKOWSKI, BENESCH and BENESCH 1949; DZIEWIATKOWSKI 1951). BOSTRÖM (1952) confirmed these findings. He also showed that injected ^{35}S sulphate in the rat and the rabbit was used only for the synthesis of sulphomuco-

polysaccharides, and that hardly any was incorporated into aminoacids (Odeblad and Boström 1952). Davies and Young (1954 a) showed that even within half an hour of its injection ^{35}S sulphate was differentially distributed among the organs of the body. After four hours incorporation followed the same pattern, and was already great. Curran and Kennedy (1955 a, b) studied the sulphate metabolism of mice, and showed that ^{35}S sulphate was concentrated in mast cells, in the mucous glands of the stomach, in intestinal Goblet cells, and in the mucus in the intestinal lumen. They also showed that the ^{35}S was in sulphomucopolysaccharides, and that most of it was intracellular. In granulation tissue around a fragment of quartz, ^{35}S was contained in fibroblasts, which formed intracellular sulphomucopolysaccharides. The authors concluded that the body could not use ^{35}S sulphate in the synthesis of sulphur-containing aminoacids, and that the traces of radioactivity which could be found in cystine must be due to its synthesis by bacteria in the intestine. Kodicek and Loewi (1955—56) studied the metabolism of ^{35}S in a wound. The greatest concentration was found between the seventh and twelfth days after the wound was inflicted, and the concentration fell to its normal level by the 23rd day. ^{35}S was incorporated into sulphomucopolysaccharides by synthesis that was enzymatic and intracellular. Destruction of the cells in vitro led to a loss of the ability of the tissue to fix sulphate. The quantities of ^{35}S fixed in the tissues were greatest in animals killed 48 hours after its injection. The radioactive substances moved like chondroitin sulphuric acid both on ionophoresis and paper chromatography. Moltke (1957) found that in dogs the uptake of ^{35}S around a wound was first increased three days after its infliction, and that the increase persisted until the seventh day. Dunphy and Udupa (1955) have claimed that in normal wound healing the concentration of hexosamine in granulation tissue rises steeply until the third day, and returns to a normal value in 8 to 12 days. Jorpes, Odeblad and Boström (1953) have shown by autoradiography that in the skin of the rat mast cells selectively take up ^{35}S, and that their maximal radioactivity is attained 48 hours after injection. More than half of the maximal degree of activity is still present 18 days after injection.

The use of ^{35}S thus provides a very good label for the study of sulphomucopolysaccharide metabolism, and for research into the tissue response to trauma and the hormonal control of tissue metabolism. Boström and Odeblad (1953) studied the effect of cortisone on the

incorporation of ^{35}S sulphate into chondroitin sulphuric acid in the rat. The concentration of sulphur in cartilage was the same in the controls as it was in the animals treated with cortisone. Radioactivity, on the other hand, was only just over half as great in the treated animals — a finding which suggested that cortisone slows the synthesis of chondroitin sulphuric acid. SPAIN, MOLOMUT and HABER (1950) claimed that during the healing of a wound cortisone caused a decrease in the concentration of polysaccharides in the area. SELYE (1953) has postulated as a general hypothesis that cortisone prevents the normal formation of a barrier of granulation tissue around an irritant substance.

2. SULPHOMUCOPOLYSACCHARIDES IN THE GASTROINTESTINAL TRACT.

LEWISON et al. (1951) measured the concentration of ^{35}S in several different tissues, and observed selective activity in the gastrointestinal tract. Several workers have since studied the location of this activity. BELANGER (1953, 1954) observed that ^{35}S in the rat's stomach was fixed in the neck cells of the glandular portion. There was also activity within the mucus that such cells contained. In the surface epithelium and in the underlying glands activity fell away sharply. Pyloric glands took up sulphate actively. The greatest activity found in the duodenum was in the Goblet cells. BOSTRÖM and ODEBLAD (1954) measured ^{35}S activity in the intestine of rabbits and mice, and found that activity within glandular cells first appeared just above the nucleus, and moved in the course of time into the mucous droplet, and so into the lumen of the gut. There was slight activity in the muscularis mucosae of the gut and in the submucosa, which persisted with diminishing intensity throughout the period of observation of 16 days. DAVIES and YOUNG (1954 b) also observed that the mucous neck cells of the stomach actively took up ^{35}S. DZIEWIATKOWSKI (1956), KENT et al. (1956) and JENNINGS and FLOREY (1956) have thoroughly investigated the synthesis and turnover of sulphomucosubstances in the gastrointestinal tract. In the guinea pig the greatest activity is found in the fovaeoli of the gastric fundus; there is little activity in the pyloric glands, but in Brunner's glands and the duodenal Goblet cells activity is marked. ^{35}S labelled methionine is not taken up by any of these

cells. The activity in rabbit's duodenal mucus starts to increase within half an hour of injection, and soon reaches its peak. ^{35}S in mucus is contained in ionic sulphate, ester sulphate and a non-ionised but dialysable fraction. Administration of cortisone does not affect the radioactivity of the mucus (KENT et al. 1956). DENKO (1958) found, on the other hand, that the giving of cortisone to hypophysectomised rats diminished the uptake of ^{35}S sulphate by the gastric glands and gastric epithelium, and that the animals developed ulcers. He concluded that cortisone caused a decrease in the rate of synthesis of sulphomucopolysaccharides in mucosa as well as in connective tissue. KOWALEWSKI and WILLIAMS (1958) studied the effect of histamine on the secretion of ^{35}S labelled mucus. 4 hours after an injection of $Na_2{}^{35}SO_4$ the concentration of ^{35}S in the gastric juice was much greater in animals which had received histamine than it was in controls. This increase was in part due to a raised concentration of labelled sulphomucopolysaccharide. In another paper the authors showed that in dogs with Heidenhain pouches there was an increase in the secretion of mucus after an injection of histamine and that for four hours after histamine was given samples continued to contain more ^{35}S than normally (KOWALEWSKI and SILBERMANN 1958). They concluded that histamine stimulates mucus secreting cells in the stomach. On the other hand, the increased rate of secretion of HCl may be more directly responsible, by a local stimulation of synthesis and secretion of mucus. KOWALEWSKI and STRUTZ (1959) observed that the concentration of ^{35}S in the gastric mucus of Shay rats was diminished if the animals had received cortisone during the month preceding the experiment.

III

PRESENT INVESTIGATION.

1. PROBLEMS CONSIDERED.

The first aim of this study is to attempt to find experimental evidence to support the hypothesis that a purely epithelial "mucous barrier" in the stomach and duodenum is not sufficient alone to explain the events which lead to ulceration: the hypothesis supposes that the deeper layers of the wall of the stomach and duodenum play an active part in defence against injurious influences.

If the mucosa is damaged mechanically recovery is rapid: this recovery is slowed when cortisone is given, since the normal mesenchymal inflammatory response — a necessity if healing is to take place, is disturbed. It is possible that such an inflammatory response is essential, and is the determining factor in the prevention and healing of ulceration. Parenteral histamine may exert either a direct or indirect effect upon the stroma and its defensive capacity. In order to clarify this problem, one may ask the following guestions:

1. To what extent does so-called ester sulphate occur in a guinea pig's stomach and duodenum.
2. Does the injection of histamine cause any change in the concentration of ester sulphate in the wall of the stomach or duodenum at a stage before the formation of an ulcer?
3. Is any such change in ester sulphate concentration localised to the epithelium or the mesenchyme?
4 Does the feeding of hydrochloric acid alone cause similar changes?
5. Can the effects of histamine be reproduced when HCl is excluded from the duodenum?
6. What effect do continuous small doses of histamine, sufficient to cause ulceration, have upon the concentration of ester sulphate in the wall of the stomach or duodenum?
7. If so, what is the usual site of the ulcer?

2. MATERIAL AND METHODS.

A. Material.

Young male guinea pigs have been used. Their weight was between 400 and 550 grams. They were kept in groups at room temperature and in ordinary cages. They were fasted for 24 hours before all experiments, but received water ad libidum. They had all been given after the test began a diet of carrots, oats and water, all ad libidum. Altogether 197 animals have been used. 39 were controls, 37 were used for studies of histamine ulceration, and 59 for studying chemically and with radioactive techniques the earlier effects of histamine. 40 animals were used to compare the effects of histamine and hydrochloric acid and of acid alone. 22 guinea pigs were subjected to pyloric ligature.

B. The preparation of the gastric and duodenal specimens.

The guinea pigs were killed by a blow on the neck. The abdomen was opened immediately and the stomach and first 5 cm of the intestine removed without any accompanying mesenters or omentum. The stomach was opened along the lesser curvature, and the gut along its antimesenteric border. The specimen was examined, and the site of any ulceration recorded. It was then cut in two close to the pyloric ring; a specimen was cut from the wall of the stomach extending 1 ½ cm orally from the pylorus; the tissue between the pylorus and the opening of the common bile duct was removed, and another specimen was taken extending 4 cm distally from the opening of the bile duct. This procedure is illustrated in Fig. 1. An attempt was made to separate the mucosa from the underlying submucosa, muscle and serosa. The latter will together be called the stroma for simplicity's sake. It is difficult in a short time to strip guinea pig's mucosa, and the following technique was devised. The specimen of duodenum was rolled out on a smooth flat piece of wood mucosa upmost. The mucosa was then scrabed off with the edge of an ordinary ampoule file. At various stages this scraping was checked against the histological appearance; with time one learnt so to scrape the mucosa that very few glands were left in the stroma and

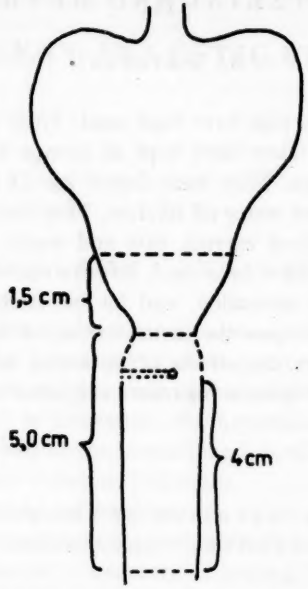

Figure 1. A drawing of the opened stomach and duodenum of the guinea pig, showing the sites from which the specimens were taken. The specimen taken from the stomach extended about 1.5 cm orally from the pylorus. The duodenal specimen extended about 4 cm aborally from the entrance of the common bile duct.

very little submucosa or muscle in the mucosal portion (Fig. 2 a). Most Brunner's glands in the guinea pig are situated just distal to the pylorus and proximal to the bile duct. Below this level they decrease sharply in number. These glands lie in the submucosa deep to the muscularis mucosae, and are included in the stroma in the analyses which will be described. Goblet cells are numerous in the mucosa distal to the opening of the bile duct: they are all found in the mucosal fraction after scraping. The stroma in the stomach includes no epithelial elements (Fig. 2 b), since the preparation of the specimen partially frees the mucosa from the underlying tissue. It is formed only by the muscle layers and the serosa. All specimens were designed to be as nearly as possible of the same wet weight, with an average of about 100 mg. The

Figure 2 a. A section of the duodenal wall distal to the entrance of the bile duct. The "mucosa" has been scraped off.

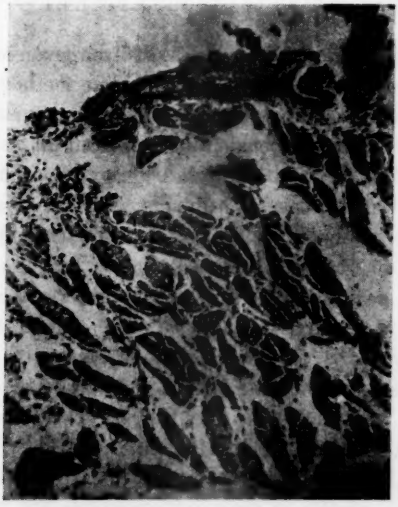

Figure 2 b. A section of the praepyloric area of the stomach. Again the "mucosa" has been scraped off. The "stroma" remains, and contains a minor part of the submucosa, the supportive layers and the serosa.

specimens were in fact weighed accurately and their weight was taken into account in the determinations made; it was necessary only that they should be of approximately the same size in order to avoid other sources of error which appear when the specimens vary greatly in mass.

The specimens were homogenised in 6 ml of water in a POTTER-ELVEHJELM glass homogeniser for a fixed period. They were first ground until no pieces could be seen with the naked eye, and then homogenised at a constant speed for a further five minutes; strictly speaking they were then a fine suspension rather than a homogenate. Samples were taken from the suspension for estimation of nitrogen content and dry weight, and for hydrolysis in formic acid. The following conventions will be used:

VM = gastric mucosa
VS = gastric stroma — as defined above
DM = duodenal mucosa
DS = duodenal stroma

C. Hydrolysis of the suspension.

Formic acid is the most suitable hydrolysing agent for use before the determination of sulphate by the benzidine method, to be described later. Formic acid was diluted to 50 %, equal volumes of the diluted acid and of the tissue suspension were placed in a thick glass tube with a ground-glass stopper. The tubes were stoppered tightly, and the stopper held down by several rubber bands. Hydrolysis took place at 100° C in an incubator. It was found that hydrolysis for 24 hours was insufficient to liberate all the sulphate.

The necessary period was determined as follows: specimens of all the tissues used in the study were pooled. The animal from which they came had received ^{35}S (by the technique generally used, which will be described later), so that the tissues were radioactive. Samples were removed after 1, 2, 3 and 4 days hydrolysis. The concentration of free sulphate was determined in each after filtering. The ^{35}S activity of the filtrate was also determined before and after adding $BaCl_2$ and centrifuging. The results of these investigations are shown in Table 1. A period of three days was chosen for hydrolysis in view of these results.

Duration of hydrolysis (days)	Chemical determination of sulphate Absorbance	Determination of radioactivity	
		Counts/min/ml of hydrolysed filtrate	Counts/min/ml of hydrolysed filtrate after adding $BaCl_2$
1	0.322	93	37
2	0.411	92	10
3	0.393	92	10
4	0.408	93	9

Table 1.

A test designed to determine the time required for hydrolysis of tissues. The test animal received $Na_2{}^{35}SO_4$, 400 μC/kg body weight, 24 hours before it was killed. A suspension was made which included all the tissues used in the study.

After hydrolysis the homogenate was filtered; samples were then taken for chemical determination of their sulphate content and for measurement of ^{35}S activity.

D. Quantitative determination.

The results of these analyses were corrected for the dry weight of the tissue. The accuracy of the dry weight determination was checked by an analysis of the nitrogen content of samples of the homogenate, using a modification of the micro Kjeldahl method. In Fig. 3 the correlation between dry weight and nitrogen is shown for 115 such analyses. DM and DS specimens have been plotted separately. The correlation coefficient r was 0.65 for DM and 0.72 for DS, both are highly significant. Dry weight has therefore been considered to be a reliable estimate of the quantity of tissue present.

E. Chemical determination of sulphate.

In choosing a method for the determination of sulphate in the suspension, it had to be remembered that only a small amount of tissue was available. A total quantity of between 1 and 10 μg was to be expected in any one sample, so that the comparatively crude gravimetrical methods could not be used.

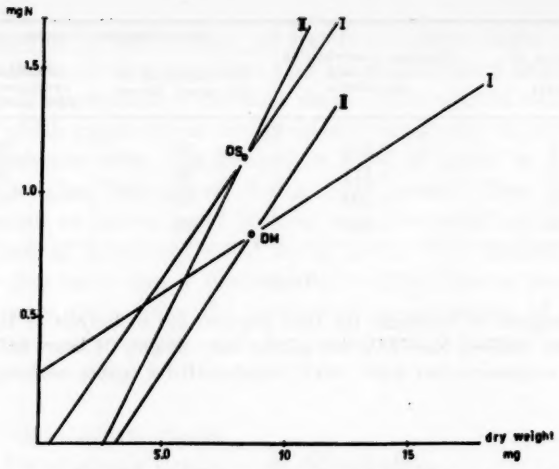

Figure 3. The relationship between the content of nitrogen and the dry weight of the duodenal "mucosa" and "stroma".

A more precise technique is the micromethod described by KENT and WHITEHOUSE (1955 a), which can measure 2 μg of sulphate within a limit of accuracy of 10 %. This method was tried in the present study, but difficulties was encountered, particularly in filtering through glass, where the process was slow and the results inconstant. The main problem was to collect all the precipitated benzidine sulphate so that the excess benzidine reagent was properly washed. The author has developed a method by which the benzidine sulphate precipitate can be separated by centrifugation (HÄKKINEN 1960). This method speeds the estimations considerably; depending upon the centrifuge used, one may make as many as 100 sulphate estimations in one day. Moreover, the least quantity of sulphate measurable is reduced by this method; the author has been able to measure amounts as small as 1.0 μg, with a standard error of 0.18 μg (as calculated from 25 duplicate determinations). In all other respects method used has followed that of KENT and WHITEHOUSE (1955 a).

Reagents.

1. 50 % formic acid (Merck pro anal.).
2. Glacial acetic acid (Merck pro anal.).
3. 96 % alcohol.
4. Acetone (Merck pro anal.) alcohol mixture 1 : 1 v/v.
5. Benzidine reagent: a 0.5 % solution of benzidine (pro anal.) in alcohol, kept in the dark in a refrigerator, and renewed regularly.
6. Barium sulphate suspension (pro anal.).
7. 1 N hydrochloric acid (Merck pro anal.).
8. 0.5 w/v % thymol (Merck pro anal.) in 2 N NaOH.
9. 0.1 N sodium nitrite solution (Merck pro anal.).

Procedure.

1 ml of the filtrate of the hydrolysed suspension, containing 25 % formic acid, is placed in a test tube. 1 ml of glacial acetic acid, 3 ml of acetone/alcohol and 1 ml of benzidine reagent are added. The tube is thoroughly shaken, corked, and left at + 4° C about 12 hours. The contents are then poured into a centrifuge tube of about 10 ml capacity which tapers to a sharp point. One drop of $BaSO_4$ suspension, previously washed free of excess barium or sulphate ions, is added, and the tube is centrifuged for about 5 minutes at 3000 r.p.m. 2 more drops of $BaSO_4$ suspension are then added, and the tube is centrifuged for a further 15—20 minutes. It is then decanted, and the mouth of the tube wiped dry with cellulose wadding. 9 ml of acetone/alcohol is added, and the precipitate is broken up with a stiff wire. It is then centrifuged for 5 minutes; further 2 drops of $BaSO_4$ suspension are added, and it is again centrifuged for 10 minutes. The added barium sulphate now forms a tightly packed cone, with a height of 1—1 ½ mm. The tube is decanted. A further 9 ml of acetone/alcohol are added, and centrifuging is repeated for a further 10 minutes. After decanting, 3 ml of normal HCl are added in order to dissolve the benzidine sulphate, and the precipitate is again broken up with a wire as before. After half an hour 2 ml of water are added, the tube is centrifuged for 5 minutes, and the solution containing benzidine hydrochloride is poured into another tube for diazotosation. Benzidine HCl is diazotised by adding 1 ml of sodium nitrite solution. After three minutes 5 ml of thymol

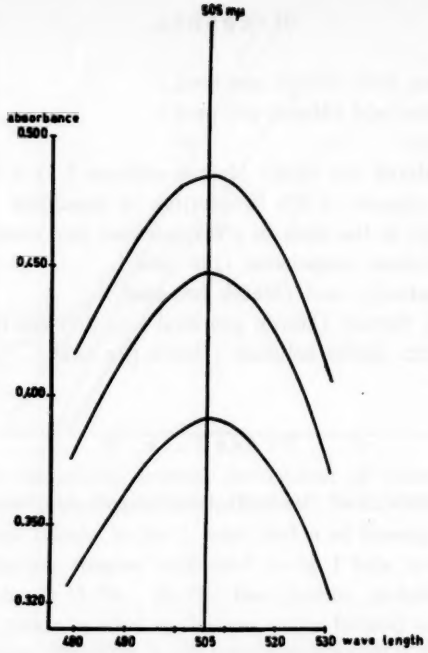

Figure 4. The absorption maximum curve for the diazo colour obtained in the determination of sulphate by the benzidine method (KENT et al. 1955 a), as measured with the Beckman DU spectrophotometer.

solution are added; the solution becomes bright red if benzidine was present. The intensity of the colour is directly proportional to the quantity of sulphate in the solution. The absorption is measured in a BECKMANN DU spectrophotometer; the absorption maximum under these conditions is 505 $\mu\mu$ (Fig. 4). According to KENT and WHITEHOUSE at least 90 % of the sulphate is precipitated by benzidine. Any error in this precipitation is minimised by the fact that all the samples to be compared have been treated in the same way.

The sulphate content was calculated from a standard graph: the relation of absorption to the amount of sulphate present is linear, at least up to 9 μg. All the estimations fell within this range. Every point on the standard (Fig. 5) was determined from at least 4 dupli-

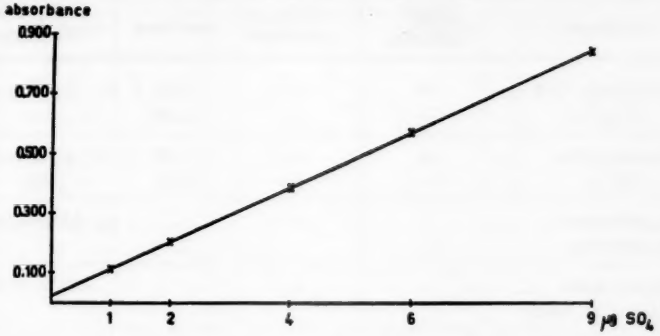

Figure 5. The standard curve used in the determination of sulphate by the benzidine method.

cate determinations. The applicability of this test to suspensions was tested by recovery, with a result of 97 %. All routine determinations were made in duplicate.

F. Sources of error in the analysis of ester sulphate.

In this study acid-hydrolysalbe sulphate has been measured. The technique of hydrolysis described above was that best suited to the subsequent determination.

a. Tests involving hydrolysis.

Faber (1949) and Kirk and Dyrbye (1956) have examined the sulphate content of human aorta after acid hydrolysis. The former author presumed that all the sulphate present was in the form of ester sulphate. His determinations were gravimetric from a barium precipitate. The latter authors describe their more accurate methods of hydrolysis and determination. Specimens were hydrolysed in concentrated HCl for 16 hours at 118° C. Chloride was removed by absorption, and sulphate was determined by a modification of the benzidine method.

Sample	Formic acid hydrolysis	HCl hydrolysis	Absorbance	—SO_4 % of dry weight
Guinea pig DM ...	+	—	0.340	
— „ — ...	—	+	0.350	
Human aorta	+	—	1.120	0.23
— „ —	—	+	1.020	0.21
Human aorta ..... (Faber 1949)	—	+		0.12—0.34
Human aorta ..... (Kirk et al. 1956)	—	+		0.212—0.308

Table 2.

The sulphate content of human aorta and guinea pig duodenum after hydrolysis in 25 % formic acid or concentrated HCl.

An analysis has been made of sulphate in human aorta after hydrolysis both with formic acid as described above and by the method of KIRK et DYRBYE with concentrated HCl. Chloride was removed by evaporating the hydrolysed filtrate in a porcelain dish, and then washing with distilled water until no precipitate appeared with $AgNO_3$. Sulphate was then measured with benzidine. A similar duplicate hydrolysis has been carried out with guinea pig duodenum. The results are compared with those of FABER and of KIRK and DYRBYE in Table 2. Both methods of hydrolysis give an identical result. The results for the aorta are comparable with those of the other authors.

b. The determination of sulphate after ashing.

Besides hydrolysis, tests have been carried out in which the suspension was evaporated in a porcelain dish, and then kept at 500° C for 2 hours. The ash was dissolved in water and sulphate determined as before. Tests were also made with a known quantity of cysteine in solution in order to ascertain whether aminoacid sulphur is oxidized during hydrolysis or ashing. The results of these tests are shown in Table 3. The same values for sulphate are obtained after ashing as after formic acid hydrolysis. The sulphur in cysteine does not appear to be

Sample	Formic acid hydrolysis	Ashing at 500°C	Absorbance
Guinea pig DS I	—	+	0.375
— „ —	+	—	0.372
Guinea pig DS II	—	+	0.208
— „ —	+	—	0.199
Cysteine 50 μg	+	—	0.020
— „ —	—	+	0.021

Table 3.

The determination of the sulphate content after ashing at 500° C or after hydrolysis in formic acid.

oxidized to sulphate under such circumstances, confirming that tissue hydrolysis does not oxidize aminoacid sulphur to sulphate. KENT and WHITEHOUSE (1955 a) have also reported that neither cysteine nor methionine affect the determination of sulphate under these conditions.

c The total sulphur content of the stomach and duodenum.

Specimens of suspension were ashed in the presence of nitric acid, perchloric acid and sodium bromide by the method of ÅGREN (1958); this treatment should cause all the sulphur present to be converted into sulphate. Values for this total sulphur, determined as sulphate, are compared in Table 4 with the sulphate content of the same tissue as determined after hydrolysis with formic acid. Some 5—10 % of the total sulphur in the tissue occurs as sulphate.

d. The phosphorus content of the stomach and duodenum.

The content of phosphorus in some of the hydrolysates was measured by the method of FISKE and ZUPPAROW (HAWK 1954), since it has been claimed that phosphorus affects the precipitation of sulphate with benzidine (KENT and WHITEHOUSE 1955 a; KIRK and DYRBYE 1956). The phosphorus content varied between 0.47 and 1.2 % of the dry weight, or 40—72 μg (per sample) in terms of sulphate (see Table 5). INGRAHAM and VISSHER (1939) has recorded the total phosphorus of a dogs gastric

Sample	Formic acid hydrolysis	Oxidizing ashing	μg —SO_4/mg of dry weight
Guinea pig VM	+	—	0.59
— „ —	—	+	6.10
Guinea pig VS	+	—	0.19
— „ —	—	+	1.95
Guinea pig DM	+	—	0.52
— „ —	—	+	7.00
Guinea pig DS	+	—	0.48
— „ —	—	+	3.25

Table 4.

The total sulphur content of the walls of the stomach and duodenum of the guinea pig, and acid-hydrolysable sulphate expressed as a proportion of the total sulphur content.

Sample	Formic acid hydrolysis	Absorbance	Phosphorus	
			μg/mg of dry weight	μg/ analysis
4 μg -SO_4	—	0.384		
70 μg P + 4 μg -SO_4 ..	—	0.380		
70 μg P	—	0.020		
Guinea pig VM	+	0.180		
„ + 35 μg P ...	+	0.194		
Guinea pig VS	+	0.147		
„ + 35 μg P ...	+	0.153		
Guinea pig VM	+		12.0	72
— „ — VS	+		4.7	40
— „ — DM	+		11.5	62
— „ — DS	+		7.2	46

Table 5.

The phosphorus content of the walls of the stomach and duodenum of the guinea pig, and the results of determinations of sulphate in the presence of phosphorus.

mucosa as 1.004—1.323 % of dry weight. A solution containing 70 μg of phosphorus was not precipitated by benzidine, and the final reading was no different from the blank. When 70 μg of phosphorus were added to a standard solution of sulphate the result of sulphate determination was not affected (Table 5). It is obvious that under the circumstances tested the presence of phosphorus does not affect the determination of sulphate.

e. Dialysis.

Separate suspensions were made of the mucosal and stromal fractions from 10 control guinea pigs and from 10 animals killed 24 hours after injection of histamine. The suspensions were dialysed against distilled water for 3 days at + 4° C. The animals had been given 400 μC of $Na_2{}^{35}SO_4$/kg body weight 24 hours before they were killed. The dialysate was concentrated and its sulphate content measured both with & without hydrolysis with formic acid. The ^{35}S activity was measured after evaporation of a volyme of dialysate which corresponded to 0.5 ml of tissue suspension inside the dialysing membrane.

No free sulphate could be found in the dialysate either before or after hydrolysis with formic acid. Radioactivity was slightly higher than background activity, but counting over 10 minutes gave a result with above the statistical upper limit of error of the technique. Certainly net activity in the dialysate was less than 1 % of the total activity of the tissue suspension. It is admitted that the method of dialysis used does not give a quite complete recovery of free sulphate.

These results justify the conclusion that the sulphate present in the tests almost all derived from undialysable, so-called ester sulphate, and was neither free inorganic or dialysable conjugated sulphate.

In summary, it has been shown that formic acid hydrolysis was adequate to liberate all sulphate that could be hydrolysed by acid. So far as is known, no sulphate is resistant to such hydrolysis. Aminoacid sulphur was not oxidized by the experimental methods used. It appears that only between 5 and 10 % of the total sulphur in the stomach and duodenum is in the form of sulphate. Any phosphorus present did not affect the determination of sulphate with benzidine. Finally, the results suggest that the sulphate in the gastroduodenal tissue can be determined with sufficient accuracy by the methods used, and that the benzidine method was not liable to error.

G. Isotope methods.

The radioactive sulphur used in this study was obtained from the Radiochemical Centre, Amersham, England, as carrier-free $Na_2{}^{35}SO_4$. Before injection into the animals 4 mg of Na_2SO_4 was added to each 100 μC. It was injected subcutaneously.

Radioactivity was determined in the same sample as was used to measure the sulphate content. It had to be taken into consideration, in forming a technique of measurement of radioactivity that the suspension of hydrolysed tissue contained solid particles, and that these were bound to cause error by absorbing soft β rays. Measurements were therefore carried out on a filtrate. Liquid also absorbs radiation (WALSER, REID and SELDIN 1953). In order to avoid such absorption, the filtrate was evaporated: as a result the degree of self-absorption was small.

1 ml samples of filtrate of hydrolysed tissue were pipetted into aluminium cups and the liquid evaporated under a lamp. Table 6 shows the effect of dilution of the same sample, and shows that the method of preparation produced suitably thin films. RAY and ARGUS (1951) and EVERETT and SIMMONS (1952) have obtained consistent results from such a method of preparing biological material for determination of radioactivity.

The measurements were made using a scaling unit (LABGEAR D 4019, and an α-β valve (FHZ 15 b, 1.40 mg/cm^2). The cup was 0.4 inches from the window. The count was measured for each cup over 10 minutes, and varied usually from 700 to 4000. Duplicate specimens were made from each tissue sample. The standard error was 3.7 counts per minute (as calculated from 25 duplicate determinations). Decay was corrected graphically.

Dilution of the sample	I Counts/min.	II Counts/min.	III Counts/min.
Original	96	113	126
Diluted 1 : 1	55	49	52

Table 6.

The effect on the netivity (in counts/minute) of dilution of three samples.

H. Preparation of the histamine.

Histamine in beeswax was prepared by the method of Code and Varco (1942). Histamine acid phosphate (BDH) was used. Histamine hydrochloride absorbs water, and is harder to treat. The final preparation had the consistence of cream, and could be expressed at room temperature through a No. O needle. All injections were subcutaneous, and a dose of 3 mg/kg body weight was used in every case. Antistin (Ciba), 20 mg/kg body weight, was given subcutaneously immediately beforehand, in order to avoid anaphylaxis, when this substance obviously had no other effects to the experiments.

I. The technique of operation.

Ligature of the pylorus was carried out as follows:

Under ether anaestesia an upper abdominal midline incision was made and the peritoneum opened. A thread was passed behind the pylorus, between the bowel wall and the mesenteric veins, and was tied tightly. Care was taken not to touch the duodenum. An incision was made in the wall of the stomach, and its edges sewn to the abdominal wall, so that a free gastric fistula was established.

J. Statistical methods.

The standard error of the difference between two independent observations x' and x" on the same material has been calculated from the formula

$$(1) \qquad \varepsilon\{x' - x''\} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_i' - x_i'')^2}$$

where n is the number of duplicate determinations. The standard error of an individual observation was calculated by division of the standard error of the difference by $\sqrt{2}$, or:

$$(2) \qquad \varepsilon\{x\} = \varepsilon\{x'\} = \varepsilon\{x''\} = \frac{\varepsilon\{x' - x''\}}{\sqrt{2}}$$

The mean $\bar{x}$ of a series of observations was calculated in the usual way from

$$(3) \qquad \bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i$$

where x_i are the observations and n their number. The standard deviation $s\{x\}$ of an observation, was calculated from the formula

$$(4) \qquad s\{x\} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

and the standard error of the mean from

$$(5) \qquad s\{\bar{x}\} = \frac{s\{x\}}{\sqrt{n}}$$

In testing the significance of the difference between two means $\bar{x}$ and $\bar{y}$, a value of t was calculated from

$$(6) \qquad t = \frac{\bar{x} - \bar{y}}{s\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}}$$

where

$$(6\,a) \qquad s = \sqrt{\frac{(n_x - 1)\, s^2\{x\} + (n_y - 1)\, s^2\{y\}}{n_x + n_y - 2}}$$

The value of t was then used to determine the value of P from Student's tables (FISHER and YATES 1957) for $n_x + n_y - 2$ degrees of freedom. When P was between 0,05 and 0.01, a difference was considered to be significant at the 5 % level. Similarly a difference was significant at the 1 % level when P was less than 0.01 but greater than 0.001, and significant at the 0.1 % level when P was less than 0.001.

The coefficient of correlation, r, has been calculated from the formula

$$(7) \qquad r_{x-y} = \frac{s_{xy}}{s\{x\} \cdot s\{y\}}$$

where

$$(8) \qquad s_{xy} = \frac{1}{n-1} \sum_{i=1}^{n_x} \sum_{j=1}^{r_y} (x_i - \bar{x})\,(y_j - \bar{y})$$

$s\{x\}$ was calculated from formula (4) and $s\{y\}$ from the same formula by substituting values of y.

In determining the degree of significance of the deviation of r from 0, a value of t was computed from

$$(9) \qquad t = \sqrt{n-2} \cdot \frac{r_{x-y}}{\sqrt{1-r^2_{x-y}}}$$

where $n-2$ are the degrees of freedom.

The value of t was used to determine the probability level from Student's tables exactly as for the difference between two means.
For the purpose of this work a difference between means or a coefficient of correlation has been considered to be almost significant (*) when $0.05 > P > 0.01$, significant (**) when $0.01 > P > 0.001$, and highly significant (***) when $P < 0.001$.

3. RESULTS.

A. Macroscopical lesions caused by histamine.

The purpose of this work was to investigate the changes that took place before ulceration occurred. The dose of histamine and the duration of its effect had thus to be decided by preliminary tests. The process had also to be followed to the stage of ulceration. The area investigated had to show a consistent reaction to histamine, and the macroscopical lesions had not to be too advanced, while constant changes in sulphate content had to occur. It was soon noticed that individual guinea pigs differ in their reaction to histamine, and that very different quantities of histamine and times of exposure were needed to cause duodenal ulceration. It was obviously undesirable to compare the sulphate content in animals with ulcers and animals whose duodenum seemed normal, since the results would be determined by different factors. For the sake of consistency the same single dose of histamine was used in all experiments; it was given so that the rate of absorption would be as nearly as possible the same in all cases. 3 mg of histamine base/kg body weight

Number of animals	No. of histamine injections	Interval after last injection	Number of animals with ulcer	Site of ulcer	
				Duodenum	Stomach
39	—		—	—	—
4	1	4 hours	—	—	—
22	1	1 days	—	—	—
19	1	2	—	—	—
7	1	3	—	—	—
7	1	4	—	—	—
4	1	5	—	—	—
2	1	7	—	—	—
2	1	9	—	—	—
3	2	2	—	—	—
4	3	1	4	4	1
3	4	1	2	2	—
3	4	2	—	—	—
2	6	1	2	2	1
6	7	1	6	6	1
1	7	1	—	—	—
2	9	1	—	—	—
3	9	1	3	3	—
2	14	5	—	—	—

Table 7.

The effect of histamine injections in causing ulcers of the stomach and duodenum of the guinea pig.

in beeswax proved to be a suitable dose. No ulcers were caused by one injection of this dose. There was no death when the antihistamine cover was used.

Table 7 shows the number of doses of histamine which were given, the duration of the experiments, and the frequency with which ulceration was found. Preliminary tests were carried out, with the intention of learning how many daily injections of histamine were needed to cause ulceration. It was found that three or more such injections regularly caused ulceration. It was obviously possible that two injections could cause ulceration, so that only one injection was given in the tests, as being unlikely to cause ulcers. There is no definite region in which ulceration always occurs, for some animals failed to develop ulcers after 4, 7 or even 9 injections. Two animals, moreover, failed to show any lesions at all in the stomach or duodenum. When ulcers occurred,

Figure 6. An acute ulcer due to histamine in the duodenum of a guinea pig.

they often perforated; by definition this happened soon after they formed. Exactly how soon cannot be told. Moreover, by the nature of the experiment, any counts of ulcer incidence were arbitrary. The animals given 3 or more injections in the preliminary tests were regarded as one group, and animals were killed as soon as they began to look ill. It appears that animals which were sensitive to histamine all developed ulcers after between 3 and 7 injection, and that there was a resistant population which would never develop ulcers. This possibility has not been followed further, as it seemed irrelevant to the rest of the work.

Most ulcers were in the duodenum. Only three animals had gastric ulcers, always on the greater curvature in the fundus. The duodenal ulcers were within 5 cm of the pylorus; the site was otherwise not determined exactly. The criterion of ulceration was a crater, with a clearcut edge, extending down nearly to the serosa on macroscopical examination. Nearly all the lesions were obviously ulcers, between 2 and 5 mm across. They were sometimes multiple, and usually circular or nearly so. Histological examination of part of them confirmed that they were acute ulcers (see Fig. 6). The same criteria were used to diagnose the gastric ulcers. They were smaller than the duodenal ulcers, and generally about 1—2 mm across. 24 to 48 hours after a single injection of histamine the mucous membrane of the duodenum often appeared oedematous, and small surfage haemorrhages could occasionally be seen.

Number of animals	Depôt histamine 3 mg/kg	Aqueous histamine mg	HCl ml	Duration of experiment (hours)	Animals with ulcer	Animals with erosions	Site of ulcer or erosions	
							Duodenum	Stomach
3	—	—	30+30	30	—	3	3	1
4	—	—	15+15	30	—	—	—	—
2	—	—	15+15 + 15+15	30	—	1	—	1
5	—	—	15+15+15 + 15+15+15	30	—	1	1	—
5	+	—	15+15	30	3	—	3	—
2	+	—	7+7	30	—	—	—	—
8	—	1.5	—	30	1	—	1	—
2	—	3.0	—	30	1	—	1	—
2	—	0.75+0.35 +0.35	—	30	—	—	—	—
2	—	— „ —	15+15	30	1	—	1	—
5	—	0.75+0.35 +0.35+1.50	15+15+15	30	1	—	1	—

Table 8.

The formation of ulcers by HCl feeding and histamine. The aqueous histamine has been given in part of cases in several doses during the experiment. The effect of feeding HCl compared with the effect of histamine in causing ulcers of the stomach and duodenum of the guinea pig. In addition to depôt histamine, injections of histamine in water were given to some of the animals.

B. Tests using histamine and HCl.

The effects of histamine on the chemical situation in the wall of the gastrointestinal tract will be discussed later in this section. Starting from the hypothesis that the changes which were found consistent with the existence of a direct traumatic effect of histamine on tissue, tests were carried out in which the injection of histamine was combined with the oral feeding of hydrochloric acid through a tube. 0.14 N HCl was used, a concentration close to the physiological findings in gastric secretion (Ivy, Grossman and Bachrach 1951). Table 8 shows the results of such

tests. The duration of the experiments was constant at 30 hours. The acid was given at constant intervals in the doses shown in the table. The first finding was that ulcers could not be caused by acid alone. Erosions occurring in the stomach were small, irregular in shape and superficial. The same was generally true in the duodenum, with the exception of the animals which had been given 30 + 30 ml; in their duodenum there was a narrow continuous streak of haemorrhagic mucosa, whose shape was quite unlike that of the lesions caused by histamine. The great majority of the lesions found were in the duodenum. HCl in a dose of 15 + 15 ml together with 3 mg/kg of histamine in beeswax caused a perforating duodenal ulcer in three out of five animals.

There was no close correlation between the frequency of ulceration and the quantity of aqueous histamine used. A small quantity caused ulceration almost as often as a larger.

However, the addition of oral HCl to a dose of histamine in beeswax too small in itself to be likely to cause ulceration alone, was effective in causing ulceration within a short period. The addition of acid caused an ulcer that did not differ from the duodenal ulcers caused by histamine alone. Whatever the proportion of acid to histamine, the ulcer tended to be in the duodenum.

C. The influence of tying off the pylorus on the frequency of duodenal ulceration.

22 animals were subjected to ligature of the pylorus and gastrostomy. 12 of them were given a single injection of histamine in beeswax. Examination of the duodenum after 24 hours showed it to be macroscopically normal in every case. The chief purpose of this operation was to compare the sulphate content of the duodenum with and without histamine, and after exclusion of the direct effect of acid.

D. The content of sulphate in the normal stomach and duodenum.

The tissue concentration of sulphate showed the following average values in 32 normal guinea pigs.

VM: $\bar{x} = 0.60\ \mu g$ —SO_4/mg tissue and $s\{\bar{x}\} = 0.05\ \mu g$ —SO_4/mg tissue
VS: $\bar{x} = 0.44\ \mu g$ —SO_4/mg tissue and $s\{\bar{x}\} = 0.04\ \mu g$ —SO_4/mg tissue
DM: $\bar{x} = 1.20\ \mu g$ —SO_4/mg tissue and $s\{\bar{x}\} = 0.05\ \mu g$ —SO_4/mg tissue
DS: $\bar{x} = 0.80\ \mu g$ —SO_4/mg tissue and $s\{\bar{x}\} = 0.04\ \mu g$ —SO_4/mg tissue

The mean content of sulphate in the stomach was exactly half of that in the duodenum, and the content of the mucosa greater than that of the corresponding stroma.

The coefficients of correlation were as follows:

$r_{DM—DS} = 0.65$
$r_{VM—VS} = 0.65$
$r_{VM—DM} = 0.45$
$r_{VS—DS} = 0.54$

Between the contents in the two layers of the same tissue there was a highly significant straight-line correlation ($r > 0$ at a level of probability of 0.1 %). There was a similar correlation between the stroma of the stomach and duodenum. For the mucosa of the stomach and duodenum correlation was significant ($r > 0$ at a level of probability of 1 %).

E. The effect of a single dose of histamine in beeswax.

The consistent results of the work described above suggested that it would be possible to detect any alteration in the content of sulphate which was caused by a single injection of depôt histamine. Any variation would soon be apparent, and the best way to measure the duration of any histamine effect would be by following the effect of a similar dose on consecutive days in different animals.

The object of the study was to find out whether histamine had any effect on total sulphate content. It was likely that all the histamine would have entered the circulation after 24 hours, and so exerted its effect, so this interval after injection was chosen.

Table 9 shows the collected results showing the influence of histamine on the content of sulphate as measured chemically. The content

Number of animals	Inj. of histamine	Interval between injection and death (days)	Tissue	$\bar{x}$ μg-SO_4/ mg of dry weight	$s\{\bar{x}\}$ μg-SO_4/ mg of dry weight	Significance of difference between normal and histamine treated tissues	r_{VM-VS}	r_{DM-DS}	r_{VM-DM}	r_{VS-DS}
32	—		VM	0.60	0.05		0.65***	0.65***	0.45*	0.54***
			VS	0.44	0.04					
			DM	1.20	0.05					
			DS	0.82	0.04					
18	1	1	VM	0.82	0.05	**	—0.014	0.60	0.07	0.26
			VS	0.55	0.03	—				
			DM	1.18	0.07	—				
			DS	1.19	0.11	***				
19	1	2	VM	0.59	0.06	—	0.29	0.06*	0.12	0.14
			VS	0.44	0.16	—				
			DM	1.26	0.09	—				
			DS	1.00	0.04	**				
6	1	3	VM	0.48	0.18	—	0.76*	0.99**	0.83**	0.85**
			VS	0.58	0.11	—				
			DM	1.07	0.16	—				
			DS	1.01	0.15	—				
6	1	4	VM	0.47	0.13	—	0.87**	0.64	0.72*	0.00
			VS	0.35	0.05	—				
			DM	0.78	0.11	***				
			DS	0.60	0.09	*				
3	1	5	VM	0.28	0.09	—	—0.89	0.80		
			VS	0.48	0.12	—				
			DM	0.90	0.18	—				
			DS	0.70	0.18	—				
4	1	7—9	VM	0.42	0.13	—	0.36	0.48		
			VS	0.25	0.04	—				
			DM	0.80	0.09	**				
			DS	0.52	0.11	**				
19	1	3—9	VM	0.43	0.08	—	0.50*	0.82***	0.63**	0.61***
			VS	0.45	0.05	—				
			DM	0.91	0.07	***				
			DS	0.72	0.08	—				

Table 9.

The sulphate content of normal tissue, compared with the content after a single injection of histamine in beeswax.

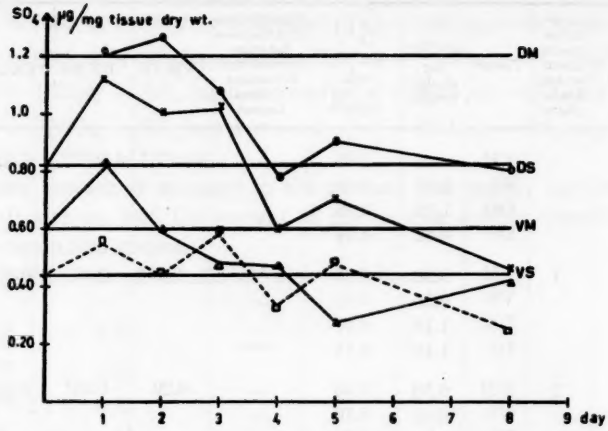

Figure 7. The variation in the sulphate content of the different layers of the wall of the stomach and duodenum which follows an injection of 3 mg/kg of depot histamine. The first values shown are those found when histamine is not given.

VM = gastric mucosa; VS = gastric stroma; DM = duodenal mucosa; DS = duodenal stroma, all obtained by the method described in the text.

has been followed until the 9th day after injection. The same results are shown graphically in Fig. 7. 24 hours after histamine injection there was a striking rise in the sulphate content of the duodenal stroma and the gastric mucosa. There was no change in the gastric stroma or the duodenal mucosa. The normal relationship between the content of various tissues vanished.

48 hours after histamine there was still a significantly increased quantity of sulphate in the duodenal stroma. The other values were little above normal. The effects were the same in all the animals during the first 48 hours. It is likely that there would have been greater individual differences during recovery, and that they would be manifest after a longer time had elapsed. For this reason only a few animals have been studied, in order to gain an impression of their progress.

4 days after histamine the sulphate content of the duodenal mucosa had decreased greatly, to below its normal level. The DS fraction had also decreased. It appeared that VM and VS were comparable to normal,

Number of animals	Doses given	Duration of experiment (hours)	Tissue	$\bar{x}$ μg-SO_4/mg of dry weight	$s\{\bar{x}\}$ μg-SO_4/mg of dry weight	Significance of difference between normal and treated tissues	r_{VM-VS}	r_{DM-DS}	r_{VM-DM}	r_{VS-DS}
9	HCl 30—60 ml	30	VM	0.51	0.13	—	0.95***	0.83**	0.36	0.88***
			VS	0.37	0.09	—				
			DM	1.04	0.18	—				
			DS	0.72	0.27	—				
7	Aqueous histamine + HCl	30	VM	0.56	0.16	—	0.71*	0.71*	0.62	0.51
			VS	0.76	0.20	—				
			DM	1.31	0.16	—				
			DS	1.13	0.15	**				

Table 10.

The effect of HCl and of HCl with histamine on the sulphate content of the walls of the stomach and duodenum of the guinea pig. The sulphate content of normal tissues is shown in table 9.

and both DM and to a lesser degree DS below control levels. If the animals killed after 3 to 9 days are considered together, the correlation coefficients devied from O significantly.

F. Analysis of sulphate content after oral HCl.

There was no significant departure from the control levels of sulphate content in the animals treated with 0.14 N HCl by the technique described. 9 guinea pigs were tested; they had received from 30 to 60 ml of HCl (see Table 10). Erosions were sometimes present to suggest that acid had had some effect. It must be emphasised that marked macroscopical changes were never found after one injection of histamine.

G. The effect of HCl and histamine on the sulphate content.

The effect of a combination of hydrochloric acid and histamine was only tested on seven animals. They were injected with histamine in water (for doses see Table 8). The results of sulphate analyses are

Number of animals	Doses given	Days after operation	Tissue	$\bar{x}$ μg-SO_4/ mg of dry weight	$s\{\bar{x}\}$ μg-SO_4/ mg of dry weight	Significance of difference from normal	$r_{DM\text{-}DS}$
12	Depôt histamine 3 mg	1	DM	1.17	0.11	—	—0.39
			DS	1.08	0.04	***	
10	—	1	DM	0.90	0.09	***	
			DS	0.88	0.11	—	

Table 11.

A comparison of the effects of ligature of the pylorus alone and of ligature of the pylorus with injection of histamine on the sulphate content of the duodenal wall of the guinea pig. Normal values are shown in table 9.

shown in Table 10. There was a statistically significant rise in the sulphate content of the duodenal stroma. The increase in the gastric stroma was not significant. The other two values were close to the control figures. The only conclusion that can be drawn is that the feeding of additional HCl does not prevent the effect of histamine.

H. The influence of pyloric ligature on the sulphate content of the duodenum after injection of histamine.

The effects of operation on the 22 animals so treated only allowed one to estimate sulphate at 24 hours; after that time the animals were too ill. The results of duodenal analyses are shown in Table 11. The content of the duodenal stroma after histamine was very high, and identical with that found in control animals 24 hours after a single injection of histamine. No gastric secretion reached the duodenum. The operation in itself did not cause any increase in sulphate content.

Number of animals	Doses given	Days after last injection	Tissue	$\bar{x}$ $\mu g\text{-}SO_4$/ mg of dry weight	$s\{\bar{x}\}$ $\mu g\text{-}SO_4$/ mg of dry weight	Significance of difference between normal and treated tissues	$r_{VM\text{-}VS}$	$r_{DM\text{-}DS}$	$r_{VM\text{-}DM}$	$r_{VS\text{-}DS}$
5	Depôt histamine 2—3 inj.	1	VM	1.07	0.14	**	0.53	0.73	0.82*	0.59
			VS	0.17	0.15	—				
			DM	1.46	0.07	*				
			DS	1.56	0.09	***				
11	Depôt histamine 4—9 inj.	1	VM	0.48	0.09	—	0.82***	0.24	0.37	0.04
			VS	0.30	0.04	—				
			DM	0.91	0.14	*				
			DS	0.50	0.07	***				

Table 12.

The effect of several injections of histamine on the sulphate content of the gastroduodenal wall of the guinea pig. The normal values are shown in table 9.

I. The effect of several injections of histamine on the sulphate content of the stomach and duodenum.

One injection of depot histamine was given daily in all cases(see Table 12). Animals killed after 2 or 3 injections showed a statistically significant increase in VM and a highly significant increase in DS by comparison with control values. After between 4 and 9 injections the sulphate content of the duodenal mucosa decreased to a level which was statistically almost significantly lower than normal. DS was highly significantly low. VM and VS did not differ significantly from normal. Table 12 shows that repeated injections of histamine caused the sulphate content of the duodenum to fall; where the ulcer occurs the sulphate content of the tissue may be said to be "exhausted".

J. Turnover tests with radioactive sulphate.

Chemical analysis of tissue sulphate shows the situation at one moment in time, and may show what net change has taken place over a period. It is, though, possible for an active process to be going on

which remains unreflected in any change in total content. Measurement of the uptake of radioactive sulphur will detect such a process. It has already been described how inorganic sulphate is esterified by an enzymatic process, and used in the synthesis of sulphomucopolysaccharides. Other injected sulphate is quickly removed from the body unchanged or in conjugates.

The turnover was examined in every case after one subcutaneous injection of $Na_2{}^{35}SO_4$. The same animals were used as were subjected to chemical sulphate analysis. The influence of histamine was measured, in the interests of clarity, after a single injection of depôt preparation.

The results of these turnover tests are shown in Table 13. A decreasing radioactivity was followed in control animals and in histamine treated animals for 4 days. Particular attention was paid to the findings of the first 4 days: after this time only single observations have been made in order to reveal the trend of the activity. The results are shown in graphic form in Fig. 8. The control animals showed a slight increase in activity from the low figure at 24 hours to a peak at the third day, after which a steady decrease occurred. There would certainly have been higher levels of activity present within the first 24 hours; most sulphate, though, is inorganic at this stage. The continuing increase until the third day must mean that the ^{35}S sulphate continues to be available in the circulation in amounts allowing its incorporation into organic sulphate. In order of activity came first the duodenal mucosa, and then the gastric mucosa and duodenal stroma. The gastric stroma never showed much activity. Injection of histamine had a profound effect in increasing activity. The increase was most marked in the duodenal mucosa, where activity rose many times over. The activity of the stromal specimen increased to a relatively smaller extent. The activity was so great in all the fractions, and its decrease after 24 hours was so fast, that the small "normal" rise until the 3rd day was perhaps concealed. On and after 4th day the treated and control animals were very alike. One must emphasize that these counts have no absolute significance; only their changes and their relative values are important.

Number of animals	Doses given	Days after injection	Tissue	$\bar{x}$ counts/min/mg tissue	$s\{\bar{x}\}$ counts/min/mg tissue	Significance of difference between normal and treated tissues	r_{VM-VS}	r_{DM-DS}	r_{VM-DM}	r_{VS-DS}
6	$Na_2{}^{35}SO_4$ 400 μC/kg	1	VM	5.6	0.3		0.77*	0.69	0.26	0.91**
			VS	2.4	0.5					
			DM	9.3	1.2					
			DS	5.0	1.0					
10		2	VM	11.4	1.3		0.50	0.82***	0.92***	0.69*
			VS	5.8	1.2					
			DM	15.8	1.8					
			DS	10.2	0.8					
6		3	VM	16.5	1.4		0.90**	0.54	0.42	0.78*
			VS	6.8	1.3					
			DM	21.0	2.0					
			DS	12.7	1.4					
6		4	VM	9.0	1.1		0.56	0.96***	0.23	— 0.60
			VS	4.2	0.5					
			DM	14.2	2.3					
			DS	9.5	1.8					
17	$Na_2{}^{35}SO_4$ 400 μC/kg + depôt histamine 3 mg/kg	1	VM	30.2	3.0	***	0.13	0.65**	0.44	0.48*
			VS	14.7	2.6	*				
			DM	54.5	6.5	***				
			DS	30.0	3.8	***				
19		2	VM	28.7	2.2	***	0.70***	0.91***	0.79***	0.79***
			VS	10.5	1.1	*				
			DM	38.7	4.1	***				
			DS	24.7	2.5	**				
7		3	VM	20.0	3.1	*	0.68*	0.66*	0.67*	0.88**
			VS	10.3	2.1	—				
			DM	28.6	4.3	—				
			DS	20.0	3.8	—				
6		4	VM	9.8	2.2	—	0.92**	0.71*	0.83**	0.91**
			VS	5.8	0.8	—				
			DM	12.0	1.8	—				
			DS	9.7	1.9	—				

Table 13.

The effect of histamine upon the rate of turnover of ^{35}S sulphate in the walls of the stomach and duodenum of the guinea pig.

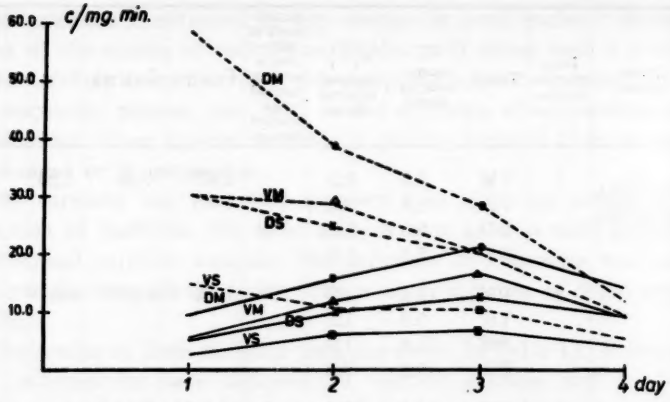

Figure 8. The turnover of ^{35}S sulphate in the different layers of the wall of the stomach and duodenum of the guinea pig. 400 μC/kg of $Na_2{}^{35}SO_4$ were injected as a single dose 24 hours before the first animals were killed. At the same time some of the animals received an injection of 3 mg/kg of depot histamine. The broken lines represent animals which were given histamine, and the continuous lines animals which were not.

IV

DISCUSSION.

Much work has been published describing attempts to estimate the sulphate content of tissue and to elucidate its metabolism. Most such work has set out to find out which tissues contain abundant sulphate. The greatest amounts have been found in cartilage, the walls of blood vessels and all forms of connective tissue. Chemical studies have recently yielded priority to studies with the isotope ^{35}S. There is very little information about tissues which contain but little sulphate — among which are numbered the walls of the stomach and duodenum (Boström and Månsson 1953). In some studies only total sulphur has been investigated. Such estimations give no idea of sulphate content, since the greater part of the sulphur is contained in cystine and methionine. Minute quantities also occur in such sulphur-containing compounds as thiamine, insulin and thiocyanate (Gilbert 1957). Sulphate, on the other hand, is combined as sulphomucopolysaccharides (Boström 1953, Dziewiatkowski 1951). This sulphate is often referred to, with reserve, as acid-hydrolysable sulphate. The reserve is due to the fact that in all tissues, however little total sulphur they contain, the content of total sulphur is at least ten times that of sulphate sulphur, and other forms of sulphate may well exist undetected. Gilbert (1957) considers that tissue sulphur is mostly contained in cystine and methionine. The wall of the stomach and duodenum are the most lively extrahepatic phenol conjugation organs, and it is not impossible that some minute quantities of phenol sulphate would exist in them (Boström 1953).

Our scanty knowledge in part reflects the difficulties met within analysing sulphur and sulphate in tissue. Most authors have used gravimetric methods, and have measured precipitates of benzidine or barium sulphate. Such methods have not permitted the measurement

of very small quantities. The methods of KIRK and DYRBYE (1956), for example, required complicated techniques of evaporation and precipitation of phosphate, and must have been time-consuming and difficult, and so unsuitable for frequent use. In the stomach and intestine analysis of sulphate has mostly taken the form of investigations of radioactive sulphate metabolism (DZIEWIATKOWSKI 1956; KENT et al. 1956). Radioactive sulphur, when injected as $Na_2{}^{35}SO_4$, has been found to be used for the synthesis of mucus in the gastrointestinal tract, and to appear incorporated into sulphomucopolysaccharides. LEVENE (1925) has succeeded in isolating mucoitin sulphuric acid from gastric mucous membrane. According to BOSTRÖM (1953) most of the sulphate in the gastric and intestinal mucosa occurs in the form of mucoitin sulphuric acid, an acid sulphomucopolysaccharide. This substance may be synthesized in mucous glands, since many investigators believe that they can synthesize acid as well as neutral mucosubstances, although the latter predominate (MEYER 1945; GROSSBERG, KOMAROW and SHAY 1950; WERNER 1953). According to KENT and WHITEHOUSE (1955 b) about 10—20 % of gastric mucosubstances occur as mucoitin sulphuric acid. The dialysis carried out in this study has shown that at least 99 % of the sulphate present was attached to molecules which were too large to pass through the membrane used: these large molecules must have been sulphomucopolysaccharides. A study of the sulphate in the tissues is different from a study of the secretions of these tissues, since the tissue only contains a small amount of the final secretory product at any one time; it was therefore to be expected that the absolute concentration of inorganic or conjugated sulphate would be low in the tissues.

There is very little information available about the normal sulphate content of the connective tissues of the gastrointestinal tract. If they are regarded as being comparable with similar connective tissue elsewhere in the body, it is likely that they will contain the same substances. SYLVÉN (1949) showed that ester sulphate, which occurs in connective tissue, is also to be found in the ground substance of rapidly growing epithelium. DZIEWIATKOWSKI (1951) and KODICEK and LOEWI (1956) have isolated ^{35}S labelled chondroitin sulphate from connective tissue, and found that in such tissue it is only chondroitin sulphate that becomes labelled when inorganic ^{35}S sulphate is given. BOYD and NEUMAN (1954) and BOSTRÖM (1953) consider the fixing of sulphate to be an enzymatic process. Destruction of the cells prevents fixation.

According to DEMPSEY and HAINES (1949) and CURRAN and KENNEDY (1955 a and b) most fixed sulphate is intracellular, and forms the ground substance of fibroblasts. A small part is extracellular, and occurs at the junctions of fibrinoid filaments (BENSLEY 1934; GLÜCKSMANN, HOWARD and PELC 1956). Tissue subjected to trauma has a high content of sulphate during healing. CURRAN and KENNEDY (1955 b), KODICEK and LOEWI (1956) and MOLTKE (1957) have studied granulation tissue, and have found that sulphate is fixed most rapidly in ulcerated tissue between 3 and 12 days after the wound is inflicted. According to DUNPHY and UDUPA (1955) the hexosamine content of a wound is at its highest after three days, and decreases to a normal level by the 12th day. A deficiency of ascorbic acid inhibits the fixation of sulphate: capillary fragility in scurvy may be due to a failure of formation of chondroitin sulphate in the capillary walls.

The turnover of ^{35}S in healthy fibrous tissue is rapid. Four days after a sigle dose very little can be detected (DAVIES and YOUNG 1954 a). In blood, muscle, liver and brain activity falls to less than 10 % of its peak level within 48 hours (BOSTRÖM 1952; DZIEWIATKOWSKI 1949). Cartilage retains more than half of its peak activity after 17 days. ODEBLAD and BOSTRÖM (1952), using autoradiography, have shown that in the fundus of the stomach and duodenum 48 hours after injection, the most intense radioactivity occurs in the mucosa. DZIEWIATKOWSKI (1956) also showed that in the duodenum the mucosa takes up ^{35}S sulphate more actively than the underlying supportive tissue. Six hours after injection the crypts showed the greatest activity. At 24 hours the tips of the villi were active. By 48 hours only a slight diffuse activity remained in the interglandular stroma and in the mucus in the lumen of the gut. Clearly sulphate plays a part in the synthesis of mucus. KENT et al. (1956), in their extensive study of the problem, pointed out that the maximal activity in the duodenal mucus occurred within about one hour after injection. Activity then fell, and roce to a new peak 5 hours after injection. At one hour only between 5 and 8 percent of the total activity derived from ester sulphate, the rest being precipitated as free sulphate ions by barium chloride. After 4 hours ester sulphate formed 69 % of the active sulphate.

KENT et al. also showed that cortisone did not affect the activity of the mucosa. If this is true, gastrointestinal epithelium differs from mesenchyme, in which cortisone slows the synthesis of chondroitin sulphate (BOSTRÖM and ODEBLAD 1953; BOSTRÖM 1953). DENKO (1958), on

the other hand, has claimed that the ability of the stomach to fix ^{35}S sulphate is reduced by giving cortisone, and suggests that the enzymatic process which leads to sulphonation is prevented both in mucosa and in connective tissue. The techniques of these authors differed, and DENKO may have been in fact measuring fixation of sulphate by mesenchyme in the wall of the stomach.

There are differences, as yet unexplained, between the fixation of ^{35}S in different parts of the intestinal mucosa. Chemical measurement of the sulphate content reveals no such differences, however (PASTERNAK, KENT and DAVIES 1958). According to JENNINGS and FLOREY (1956) ^{35}S in the small intestine is mostly taken up by Goblet cells. Activity is also found in Brunner's glands in the submucosa of the duodenum. In my material the proximal duodenum, where most Brunner's glands occur, was not included in the specimens examined. The distal duodenal mucosa contains many Goblet cells, and one may assume that they were responsible for the greater part of the radioactivity of the mucosa. In the stroma a slight diffuse acitivity might arise from the submucosa, and to some extent in the few Brunner's glands present. In the mucosal specimen from the stomach part of the activity was probable localised to the pyloric glands. The mucosal specimen here contains the submucosa as whole; some part of the activity might be surely located to these connective tissue elements. The specimens of gastric stroma, the muscle and the serosa contained only a slight diffuse activity.

After an injection of depôt histamine radioactivity and chemical content are no longer related in the duodenal mucosa. The ^{35}S activity rises sharply, while the sulphate content remains unalterated. One may conclude that the mucosa is secreting more actively and that sulphate is both taken up and secreted more rapidly than before. In the duodenal stroma ^{35}S activity and chemical content both increase. Part of the rise may be due to increased secretion. The rise in chemical sulphate is probably not due to synthesis in the basal mucosa or Brunner's glands, but to synthesis in the connective tissue.

Hydrochloric acid was given both alone and with histamine. Only chemical analysis was carried out. In the duodenal stroma the sulphate content rose when both acid and histamine were given, but failed to rise when acid was given alone. This finding suggests that the rise in the stromal sulphate content was due directly to the histamine, and not to HCl in the duodenal lumen. The effect of ligation of the pylorus,

which entirely excludes acid from the duodenum, confirms this suggestion.

In the gastric mucosal specimen it appears that histamine caused both enhanced secretion and a direct effect on connective tissue; this would not be surprising, since the mucosa sample contains both glandular and connective tissue elements. In the gastric stroma basal activity was small. Histamine caused some increase in radioactivity, but no detectable increase in sulphate content.

The effects of histamine were long-lasting, although no ulceration occured. 3 days after the initial incease in sulphate content there followed a decrease to a subnormal level, which lasted throughout the 9 days during which the animals were observed. Later observations were admittedly scanty, but it seems that this "exhaustion" of sulphate was most marked in the duodenum, the site where ulceration occurred under the conditions of this study. This reaction may be an indication of the defensive power of the connective tissue — as elsewhere in the body in the presence of a wound. One cannot press the comparison too far, since the tissue "surrendered" suddenly and perhaps too easily. No further increase in sulphate occurred with ulceration. It is of course very hard to reproduce mild ulcers proceeding to chronicity in the guinea pig, since persistence of the stimulus to ulceration usually leads to perforation. It would be advisable to follow the inception of an ulcer and the development of chronicity in less sensitive animals. It is possible that "exhaustion" coincides with ulceration, and that a rise in sulphate content, albeit slight, occurs when a balance, and so chronicity, is achieved.

The results of this study do not show what stimuli normally cause mucus to be secreted. Ivy, Grossman and Bachrach (1951) said that secretion followed direct contact of the mucosa with acid. In the duodenum we have made a separate study of this problem (Hartiala, Ball and Häkkinen). Guinea pigs were subjected to ligation of the pylorus, and the uptake of ^{35}S sulphate by the duodenal mucosa was compared in control animals and in animals receiving a single injection of depot histamine. Control values were identical to those reported in normal unoperated animals in this thesis. Injection of histamine caused an increase in uptake in both mucosa and stroma: this increase was, though, very much smaller than that which occurred in unoperated animals — about 40 % over the peak activity in the mucosa, and an increase by a half in the stroma. This change in the stroma may well

correspond to the action of histamine itself on the synthetic activity of connective tissue. Alternatively, the secrection of mucus may be stimulated in two ways, both by histamine and by the presence of acid in the duodenal lumen.

There is some information to be found in the literature about the usual sites of peptic ulcers in guinea pigs. HAY et al. (1942) gave between 2 and 11 intramuscular injections of histamine in beeswax: six animals developed ulcers, five in the duodenum and two in the stomach. Two animals who received 2 and 5 injections did not develop ulcers. MERKEL (1942) injected histamine in water. In an acute experiment the animals all developed erosions; in the chronic test 93 % did so, and one erosion appeared to be becoming chronic. All the lesions were in the stomach, and the duodenum appeared normal. MARKS (1957) found no duodenal ulcers in guinea pigs which received 10 mg/kg of histamine in beeswax three times in the week for several weeks. Some animals did develop erosions or ulcers in the fundus of the stomach. KOWALEWSKI and BAIN (1954) gave histamine intramuscularly in a dose of 100 mg/kg; within 24 hours 147 gastric and 18 duodenal ulcers resulted. Gastric ulcers were formed sooner than duodenal. Elsewhere (KOWALEWSKI 1954) the same author has claimed that gastric and duodenal congestion and ulceration follow the giving of 75 mg/kg of histamine. HOEVEN (1956) stated that histamine in beeswax caused lesions in the acid-bearing area of the stomach and in the duodenum, and that duodenal perforation occurred within 48 hours. Referring to the effects of giving hydrochloric acid, he suggested that a short exposure caused gastric ulcers and chronic duodenal. WILLIAMS (1951) also found that in under 15 hours after histamine in beeswax, 2—12 mg, more gastric than duodenal ulcers resulted. WATT (1959), also referring to a short term experiment, only mentioned gastric ulcers, which he found mainly on the greater curvature in the upper third of the stomach.

My experiments most resembled those of HAY et al, and HOEVEN, and my results are similar to theirs. The other experiments mentioned differed from mine in that the dose of histamine was much larger or its absorption faster; it was in such experiments as these that gastric ulcers were seen.

From my own observations and from the literature it is possible to conclude that guinea pigs may be caused to develop either gastric or duodenal ulcers, depending upon the method of administration of histamine, the dose used, and the rate of its absorption. Rapid absorption and

a high concentration in the blood cause gastric ulcers; slow absorption and a slightly increased blood level cause duodenal ulcers.

If one takes into consideration the possible damage to connective tissue caused by histamine, one may suggest to explain these findings on the following way, too: resistance to such damage is greatest in the stomach, and that a quantity of circulating histamine sufficient to break down this resistance will inhibit the secretion of acid, and prevent enough entering the duodenum to cause ulceration there.

V

SUMMARY.

The duodenum distal to the papilla Vateri and the praepyloric part of the stomach of guinea pig were prepared to form mucosal and stromal samples. The duodenal stroma samples included some remains of the basal mucosal glands, submucosal elements, the muscular layers and the serosa, while the gastric stroma samples were formed only by the muscular layers and the serosa.

1. The quantity of sulphate hydrolysable by acid has been measured in these specimens of the duodenal and gastric wall.

2. The subcutaneous injection of 3 mg of a depôt preparation of histamine base/kg body weight caused a significant rise in the sulphate content of the stromal part of the duodenal wall and of the mucosa of the stomach. When during the same experiments radioactive sulphate was injected in a dose of 400 μC/kg, histamine caused a rise in activity which was greatest in the mucosa of the duodenum, rather smaller in the stroma of the duodenum and the mucosa of the stomach, and very small in the gastric stroma. In this last tissue no increase occurred in the content of sulphate as measured chemically.

3. It was concluded that the changes which took place in the duodenal mucosa represented secretory activity. The changes in the mucosa of the stomach and in the stroma of the duodenum were considered to represent both secretory activity and in part a synthesis of connective tissue.

4. The administration of hydrochloric acid caused no change in the content of sulphate of any tissue.

5. In the duodenal stroma the content of sulphate rose as a result of histamine injection in animals in which the pylorus had been tied, and any direct effect of acid in the duodenum excluded.

6. Injections of histamine repeated for more than 3 days do not cause

an increase in the sulphate content of the wall of the stomach and duodenum: a decrease to below normal levels is caused, which takes place at the time when duodenal ulcers occur.

7. Ulcers caused by small single doses of histamine localised almost all in the duodenum.

8. The mechanism of the formation of experimental histamine ulcers is discussed in the light of the results presented.

The stimulation of acid secretion by histamine cannot solely explain the mechanism of histamine ulcer formation. In the present study it has been shown that histamine also has other effects upon the gastro-duodenal wall: the initial irritating or stimulating effect on both epithelial secretory and mesenchymal defence mechanism, followed by the damage and exhaustion and, if strong enough, by the formation of an ulcer.

VI

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